

FIGURE 1A

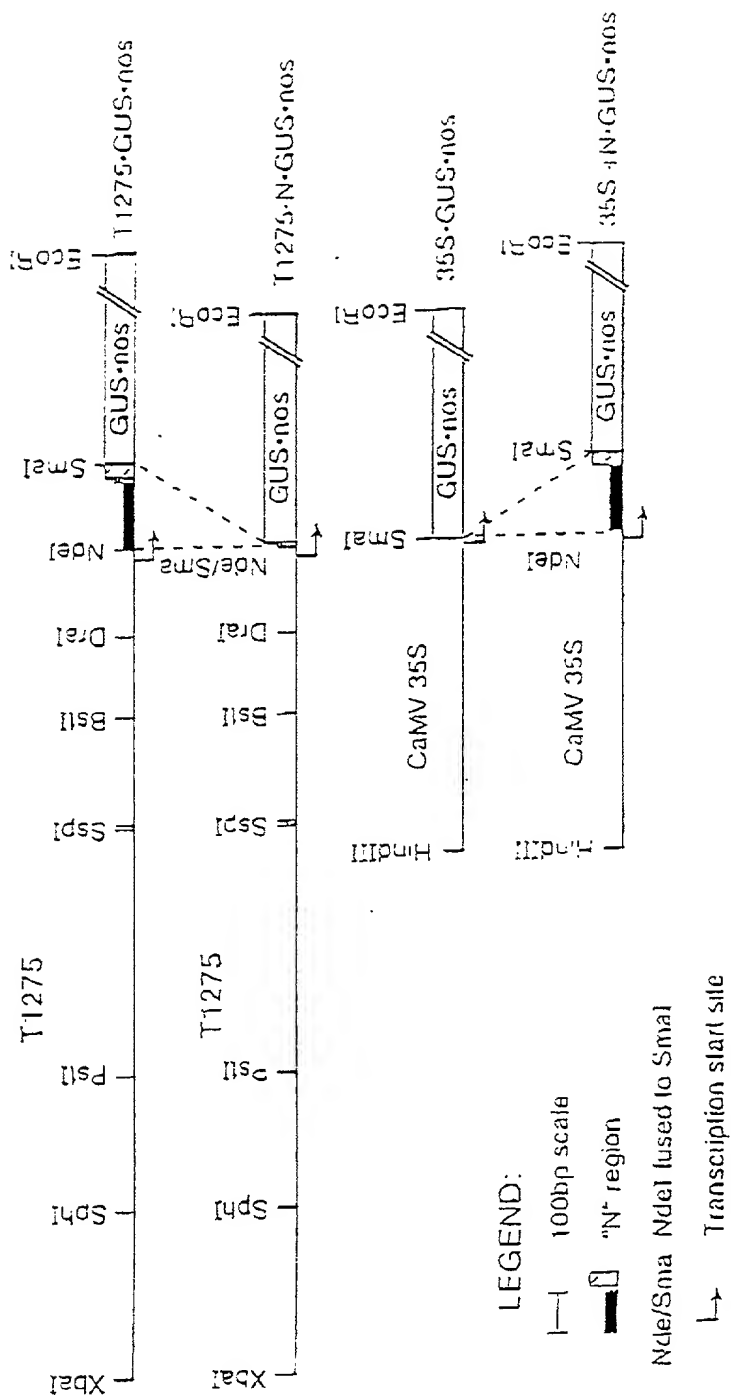


FIGURE 1B

		10	20	30	40	50
tCUP	1	-----				
REnt1.	1	-----A-----ATTGTAAGCG GGATAACAAT				
REnt2.	1	-----AT GTTGTGTGGA ATTGTGAGCG GGATAACAAT				
REnt3.	1	TT-----				
REnt5.	1	-----GGA ATTGTGAGCG G-ATAACAAT				
REnt7.	1	TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG G-ATAACAAT				

		60	70	80	90	100
tCUP	51	-----				
REnt1.	51	TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT TTTAATACGA				
REnt2.	51	TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA				
REnt3.	51	-----				
REnt5.	51	TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATANGA				
REnt7.	51	TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA				

		110	120	130	140	150
tCUP	101	-----TTA TAATTACAAA ATTGATTCTA GTACTTTTAA				
REnt1.	101	CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTTAA				
REnt2.	101	CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTTAA				
REnt3.	101	-----GATTCTA GTTTTTTTTAA				
REnt5.	101	CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTTAA				
REnt7.	101	CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTATA GTACTTTTAA				

FIGURE 1C1

	160	170	180	190	200	
cCUP	151	TTTAATGCTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTTGTTTTCA
200						
RENT1.	151	TTTAATATTT	TTACATTATT	AATTAATTTA	GAAGTTTAA	TTTTTTTTCA
200						
RENT2.	151	TTTAATATTT	ATACATTATT	AATTAACTTA	GTACTTTCAA	TTCGTTTTCA
200						
RENT3.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTTCTTTCAA	TTTGTTTTCA
200						
RENT5.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTTGTTTTCA
200						
RENT7.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GCCTTTTCAA	TTTATTTTCA
200						

	210	220	230	240	250	
cCUP	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT1.	201	GAAATCATT	TACTATTTTT	-ATAAAAACA	AAAGGGAAAA	GTGGTTATTT
250						
RENT2.	201	AAAATTATTT	TACTATTTTT	TGTAATAATA	AAGGGAGAAA	ATGGCTATTT
250						
RENT3.	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT5.	201	GAAATCATT	TACTATGGTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT7.	201	GAAATCATT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						

	250	270	290	290	300	
cCUP	251	AAATACTAGC	-CTATTTTAT	TTCAATTTTA	GCCTAAAATC	AG-CCCCAAT
300						
RENT1.	251	AAATACTAGC	CCTATTTTAT	TTCAATTATA	GCCTAAAATC	AGCCCC-AAT
300						
RENT2.	251	AAATACTAGC	CCTATTTTAT	TTCAATTTTA	GCCTAAAATC	AGCCCCCAAT
300						
RENT3.	251	AAATACCAGC	CCTATTTTAT	TTCAATTTTA	ACCTAAAATC	AGCCCC-AGT
300						
RENT5.	251	AAATACTAGC	CCTATTTTAT	TTCAATTTTA	GCCTAAAATC	AGCCCC-AAT
300						
RENT7.	251	AAATACCAAC	ACTATTTTAT	TTCAATTTTA	GCCTAAAATC	AAACCC-AAT
300						

FIGURE 1C2

	310	320	330	340	350	
CCUP	301	TAGCCCCAAT	TTCAAATTCA	AATGGTCCAG	CCCAATTCCCT	AAA-TAACCC
350						
RENT1.	301	TAACCCCAAT	TCCAAATTCA	AACGGGCCAG	CCCAATTCCCT	AAAATGACCC
350						
RENT2.	301	TAACCCCAAT	TTCAAATTCA	AATGGGACAG	CCCAATTCCCT	AAAATAACCC
350						
RENT3.	301	TAGCCCC---	-----A	AACGGGCCAT	CCCAATTCCCT	AAAATAACTC
350						
RENT5.	301	TAACCCCTAT	TTCAATTCA	AACGGGCTAG	CCCAGTTCCCT	AAAATAACCC
350						
RENT7.	301	TAACCCC---	-----A	AACGGGCCAG	CCCAATTCCCT	AAAACAACCC
350						

	360	370	380	390	400	
CCUP	351	ACCCCTAACC	C-----	----GCCCCG	TTTCCCCTTT	TGATCCAGGC
400						
RENT1.	351	GCTCCTAACC	CGCTTTTCCA	ACCCGCCCCG	TTTCCCCTTT	TGATCCAGGC
400						
RENT2.	351	GCCCCTAACC	CTCTTATCCA	ACCCACCCGA	TTTCCCCTTT	TGATCCAGGT
400						
RENT3.	351	GCCCCTAACC	CGCTTATCCA	ACCCGCCCCG	TTCCC-CTTT	TGATCCAGGC
400						
RENT5.	351	TCCCCTAACC	CGCTTATCCA	ACCCGCCCTG	TTTCCCCTTT	TGATCCAGGC
400						
RENT7.	351	GCCCCTAACC	CGCTTATCCA	ACCCGCCCGA	TTTCCCTTTT	TGATCCAGGC
400						

	410	420	430	440	450	
CCUP	401	CGTTGATCAT	TTTGATCAAC	GCCCAGAATT	TCCCCTTTTC	CTTTTTTAAT
450						
RENT1.	401	TGTTGATCAT	TTTGATCAAC	GGCCAGAATT	TCCCCTTTTC	--TTTTTAAT
450						
RENT2.	401	TGTTGATCAT	TTTGATCAAC	GACCAGAATT	TCCCCTTTTC	TGTTTTTAAT
450						
RENT3.	401	CGTTGATCAT	TTTGATCAAC	GACCAGAATT	TCCCCTTTTC	-TTTTTAAT
450						
RENT5.	401	CGTTGATCAT	TTTGATCAAC	GACCAAAATT	TCCCCTTT-C	CTTTTTTAAT
450						
RENT7.	401	CGTTGATCAT	TTTGATCAAC	GGCCAGAATT	TCCCCTTTTC	-TTTTTTCAT
450						

FIGURE 1C3

	460	470	480	490	500	
LCUP	451	TCCCAAACAC	C-CCTAACTC	TATCCCATT	CTCACCAACC	GCCACATATG
500						
RENT1.	451	TCCCAAACAC	CCCCCAACCT	TATCCCGTTT	CTCACCAACC	GCCAGATCT-
500						
RENT2.	451	TCCCAAACAC	CCCCCAACCC	TATCCCATT	CTCACCAACC	GCCAGATCT-
500						
RENT3.	451	TCCCAAACAC	CGCC-AAACC	TATCCCATT	CTCACCAACC	GCCAGATCTA
500						
RENT5.	451	TCCCAAACAC	CCCC-AAACC	TATCCCATT	CTCACCAACC	GCCAGATCT-
500						
RENT7.	451	TCCCAAACAC	CCCC-AAACC	TATCCCATT	CTCACCAACC	GCCAGATCTA
500						

	510	520	530	540	550	
LCUP	501	AATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						
RENT1.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						
RENT2.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						
RENT3.	501	I--CCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						
RENT5.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						
RENT7.	501	T--CCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA	CCCTAGCAGC
550						

	560	570	580	590	600	
LCUP	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GAATACATGG	CTTCTCAAGC
600						
RENT1.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG	CCTCTAGAG-
600						
RENT2.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG	CCTCTAGAG-
600						
RENT3.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG	CCTCTAGAGG
600						
RENT5.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG	CCTCTAGAG-
600						
RENT7.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG	CCTCTAGAGG
600						

FIGURE 1C4

	610	620	630	640	650
LCUP	601	CGTGGAAACC	TTATACTCAC	CTCCCTTTGC	TCTTACAGTA CTC-GGCCGT
650					
RENT1.	601	---GATCCCC	GGGTGGTCAG	TCCCTTATG-	--TTAC----GT
650					
RENT2.	601	----GA----	-----	-TCCCCG---	-----GGTG GTC-AGTCCC
650					
RENT3.	601	ATC----CCC	GGGTGGTCAG	TCCCTTATGT	NA-----CG NCCTAAATGN
650					
RENT5.	601	---GATCCCC	GGGTGGTCAG	TCCCTTATG-	--TTACG-----
650					
RENT7.	601	ATC----CCC	GGGTGGTCAG	TCCCTTATGT	TA-----CG TCCTN-----
650					

	650	670	680	690	700
LCUP	651	CGACCGCGGT	ACCCGGG...	.....	.....
700					
RENT1.	651	C-----	--CTNAA...	.....	.....
700					
RENT2.	651	TTAT-GTG--	---CGTC...	.....	.....
700					
RENT3.	651	CCGNCCTGNN	NNNNN-C...	.....	.....
700					
RENT5	651	-----	TCC-----	.....	.....
700					
RENT7.	651	-----	-----	.....	.....
700					

FIGURE 1C5

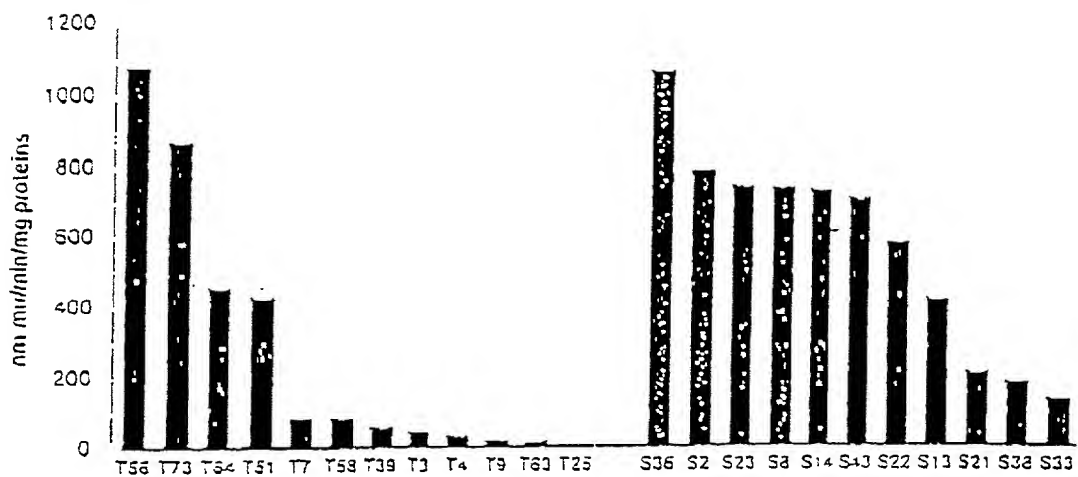


FIGURE 2A

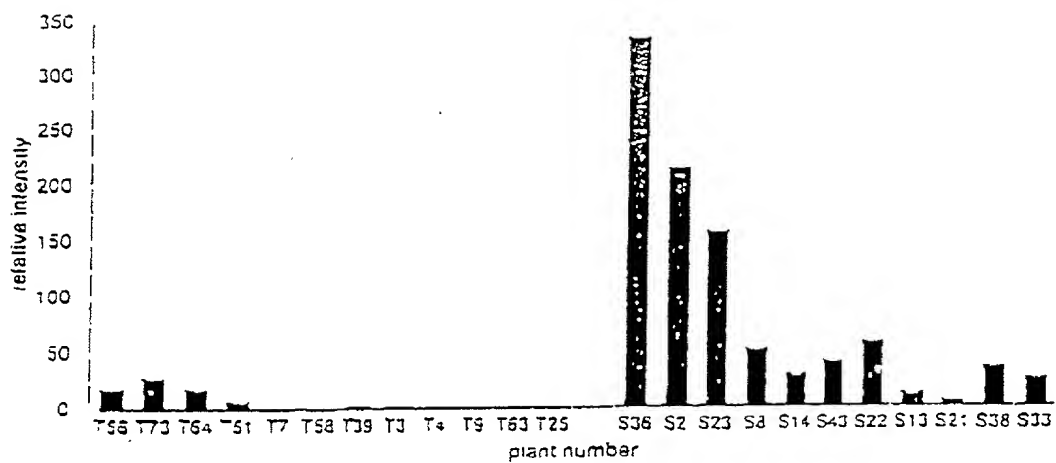


FIGURE 2B



SRI T56 T64 T58 T9 S36 S14 S13 S33 E.c.

-109

-80

-51

**FIGURE 2C**

RNA level (relative intensity)

TI275-GUS-nos

TI275-N-GUS-nos

FIGURE 3A

FIGURE 3B

**FIGURE 3C**

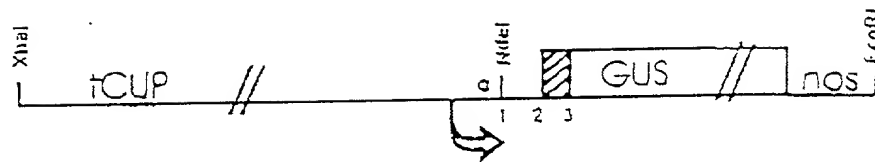
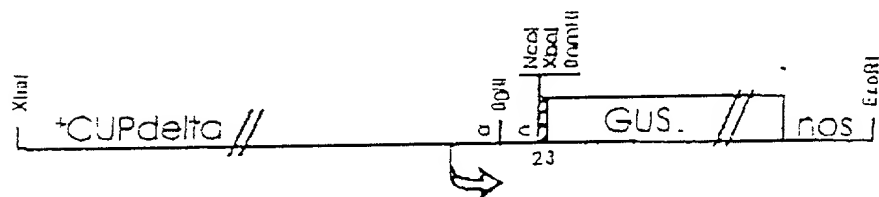


FIGURE 4A



<sup>a</sup> NdeI position +30 ACA TAT GAA  
<sup>b</sup> BglII position +29 ACA GAT CT  
<sup>c</sup> NdeI position 0 CC ACC ATG GCC TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG  
 tCUP initiation site GAA TAC ATG G / ... tCUP leader ... / CCG GGT GGT CAG TCC CTT ATG  
 Kazak consensus CC ACC ATG G

FIGURE 4B

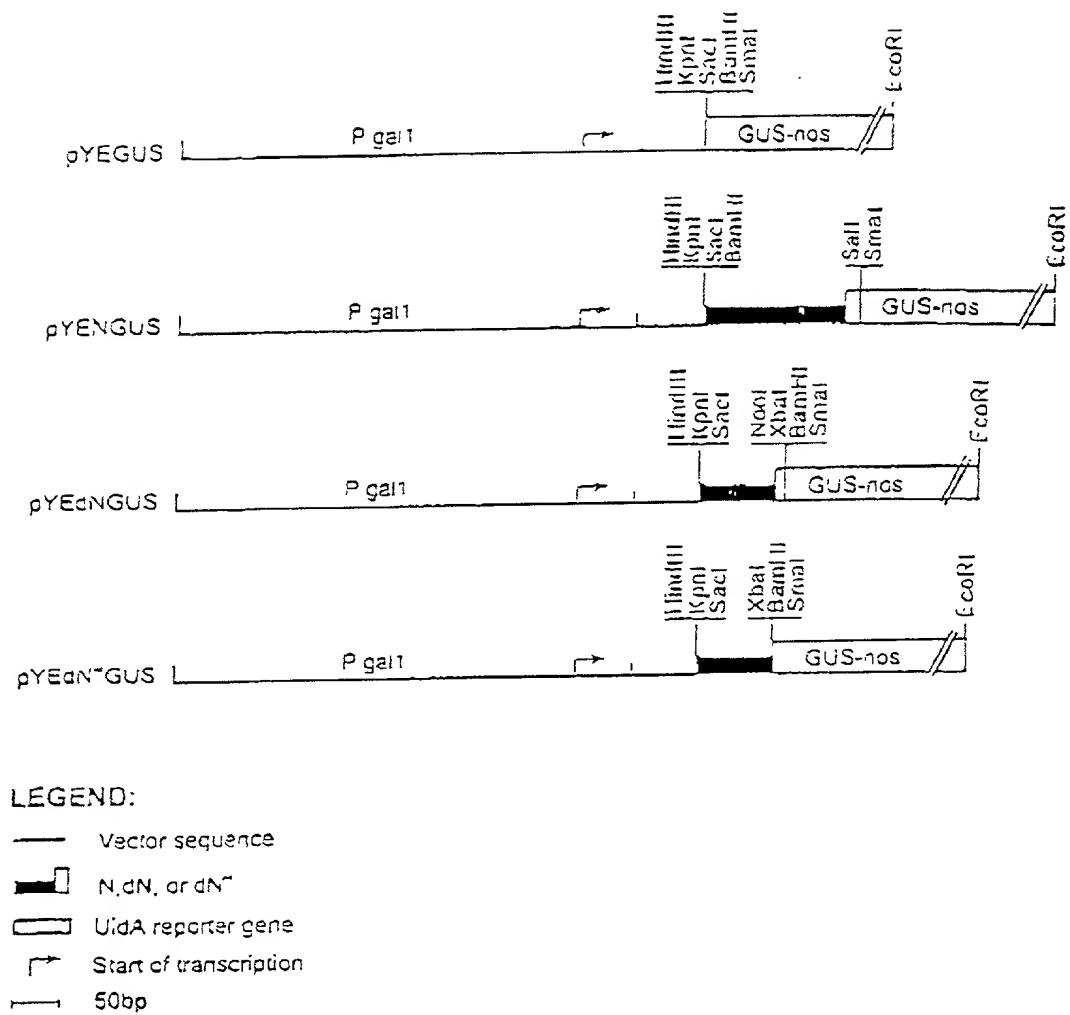


FIGURE 5A

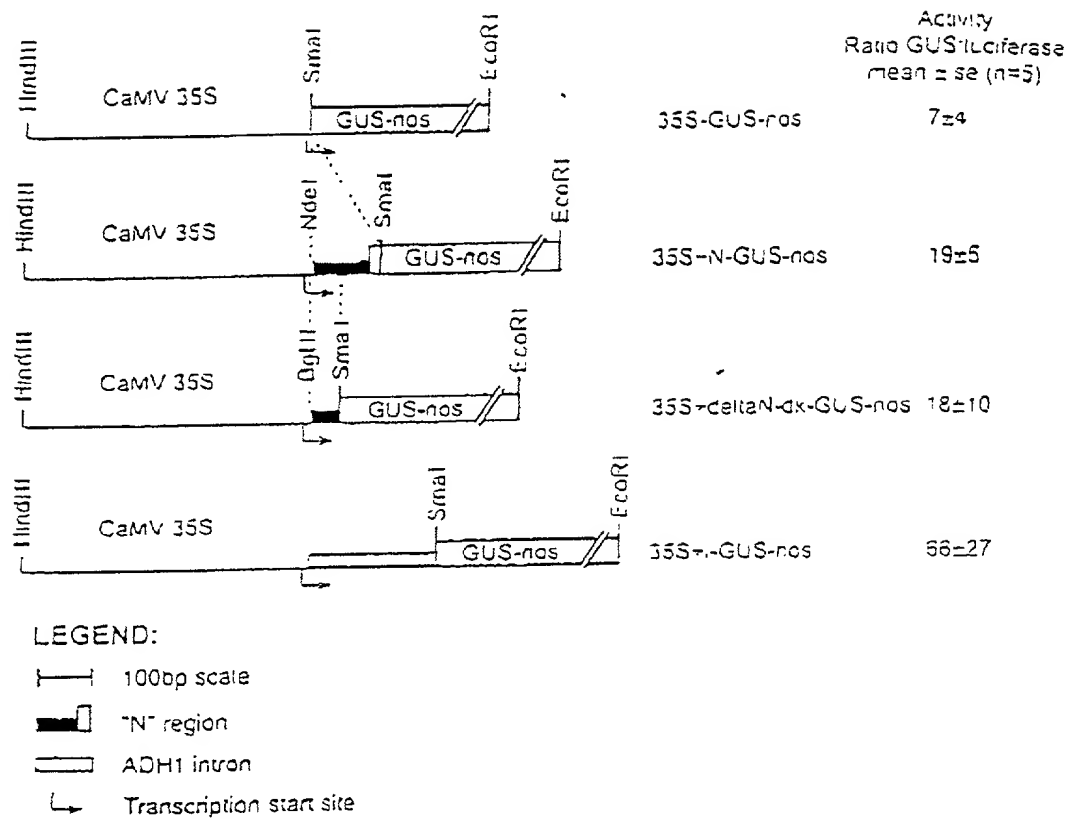


FIGURE 5B

White Spruce Callus  
Transient Analysis 14/12/99

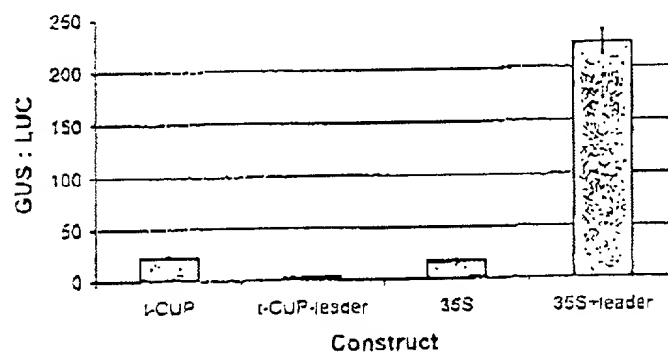
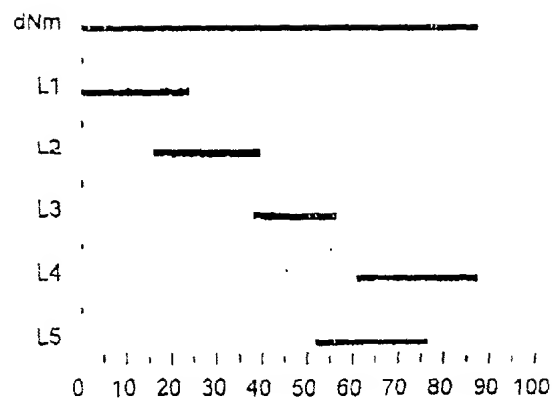


FIGURE 5C



**FIGURE 6A**

Linker 1: GGATCTATCCTCTTATCTCTCAA  
 Linker 2: ATCTCTCAAACCTCTCTCGAACCTT  
 Linker 3: TTCCCCTAACCCCTAGCAG  
 Linker 4: ATCATCCTCACCTCAAAACCCACC  
 Linker 5: AGCCTCTCATCATCCTCACCTCAA

**FIGURE 6B**



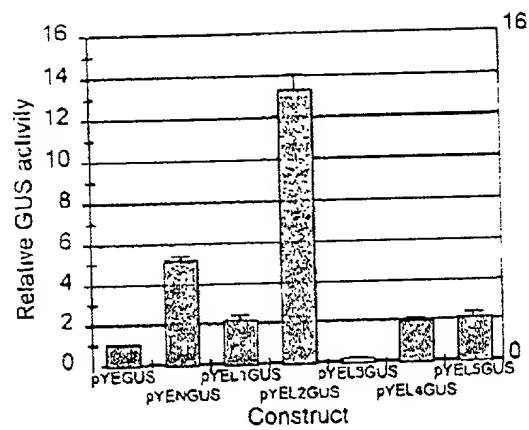


FIGURE 6C

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L2 AUCUCUCAAA**ACUCUCUC**GAACCUU  
 L2C AUCUCUCAAAACUCUCU  
 L2R ACUCUCUCGAACCUU

---

FIGURE 6D

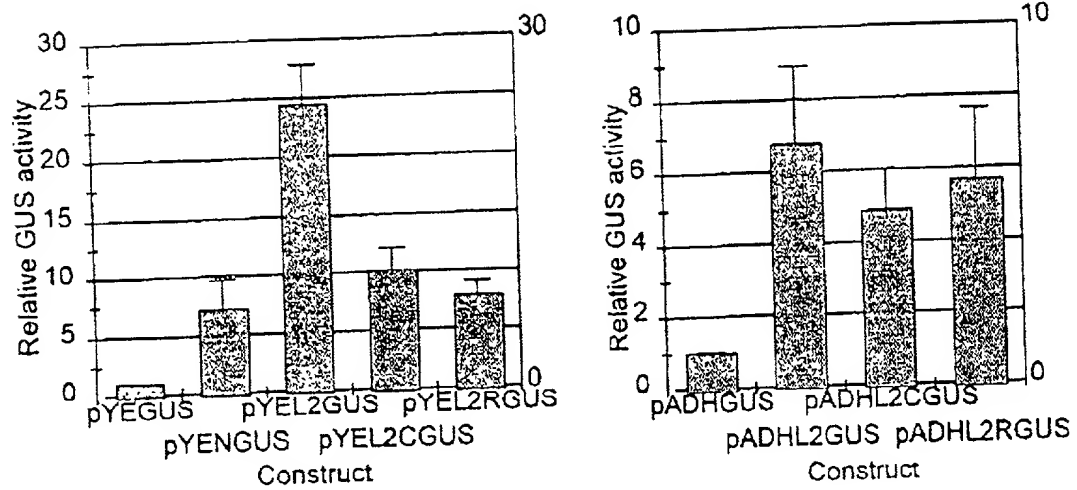


FIGURE 6E

L2	A	TCT	CTC	AAA	CTC	TCT	CGA	ACC	TT
SCAN1	a	AGA	ctc	aaa	ctc	tct	cga	acc	tt
SCAN2	a	tct	GAG	aaa	ctc	tct	cga	acc	tt
SCAN3	a	tct	ctc	GGG	ctc	tct	cga	acc	tt
SCAN4	a	tct	ctc	aaa	GAG	tct	cga	acc	tt
SCAN5	a	tct	ctc	aaa	ctc	AGA	cga	acc	tt
SCAN6	a	tct	ctc	aaa	ctc	tct	GCT	acc	tt
SCAN7	a	tct	ctc	aaa	ctc	tct	cga	GAG	tt

FIGURE 6F

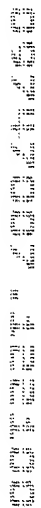
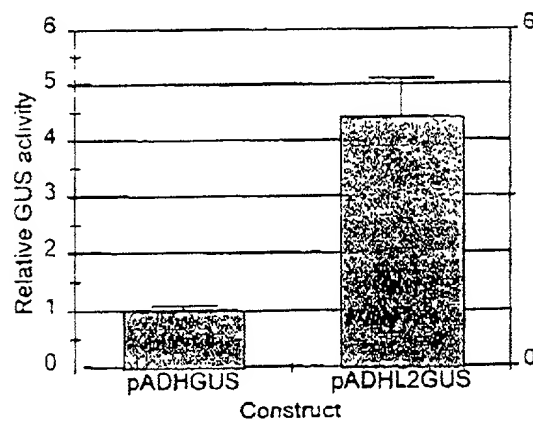
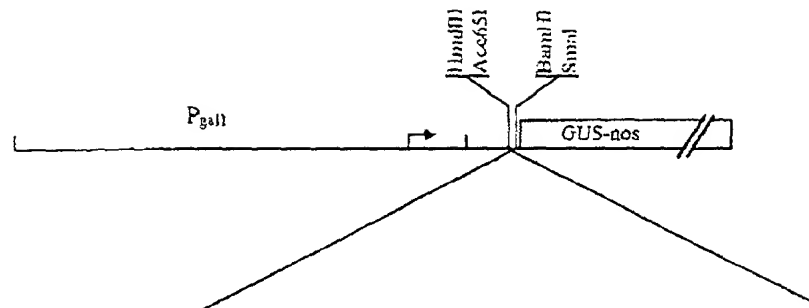
[illegible]

FIGURE 6H



pYEGUS	(no sequence)
pYEL1GUS	GGATCTATCCTCTTATCTCTCAA
pYEL2GUS	ATCTCTCAAACCTCTCTCGAACCTT
pYEL3GUS	TTCCCCTAACCCTAGCAG
pYEL4GUS	ATCATCCTCACCTCAAAACCCACC
pYEL5GUS	AGCCTCTCATCATCCTCACCTCAA
pYEL2CGUS	ATCTCTCAAACCTCTCT
pYEL2RGUS	ACTCTCTCGAACCTT
pYELMGUS	ACTCTCTC
pYESCAN1GUS	AAGACTCAAACCTCTCTCGAACCTT
pYESCAN2GUS	ATCTGAGAACTCTCTCGAACCTT
pYESCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pYESCAN4GUS	ATCTCTCAAAGAGTCTCGAACCTT
pYESCAN5GUS	ATCTCTCAAACCTCAGACGAACCTT
pYESCAN6GUS	ATCTCTCAAACCTCTCTGCTACCTT
pYESCAN7GUS	ATCTCTCAAACCTCTCTCGAGAGTT
pYEB1-L2GUS	ATCTCTCAAACCTATCTCGAACTT
pYEB7-L2GUS	ATCTCTCAAACCTCTCTCAAACCTT
pYEL2D1GUS	ATCTCTC---CTCTCTCGAACCTT
pYEL2D2GUS	ATCTCTCAAACCTCTCTCGA---TT
pYEL2D3GUS	ATCTCTC---CTCTCTCGA---TT
pYEL2L2GUS	ATCTCTCAAACCTCTCTCGAACCTTTCTCTCAAACCTCTCTCGAACCTT

#### LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 6I

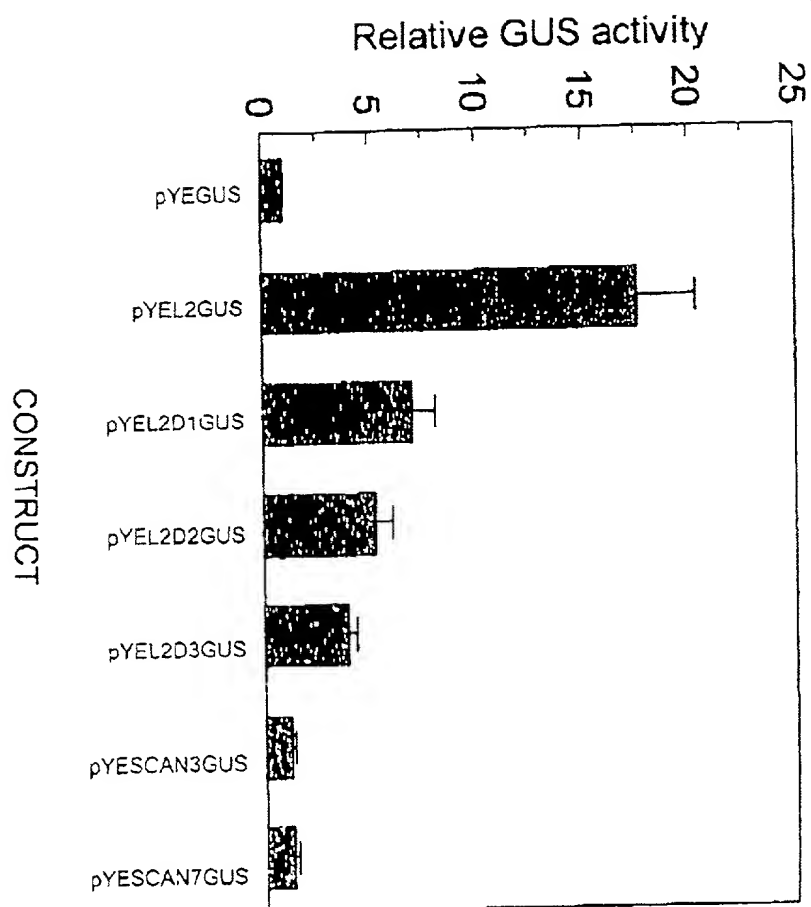


FIGURE 6J

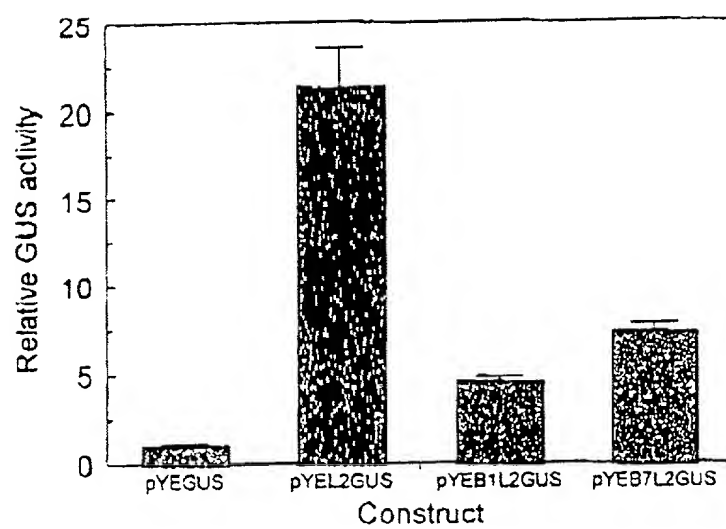


FIGURE 6K

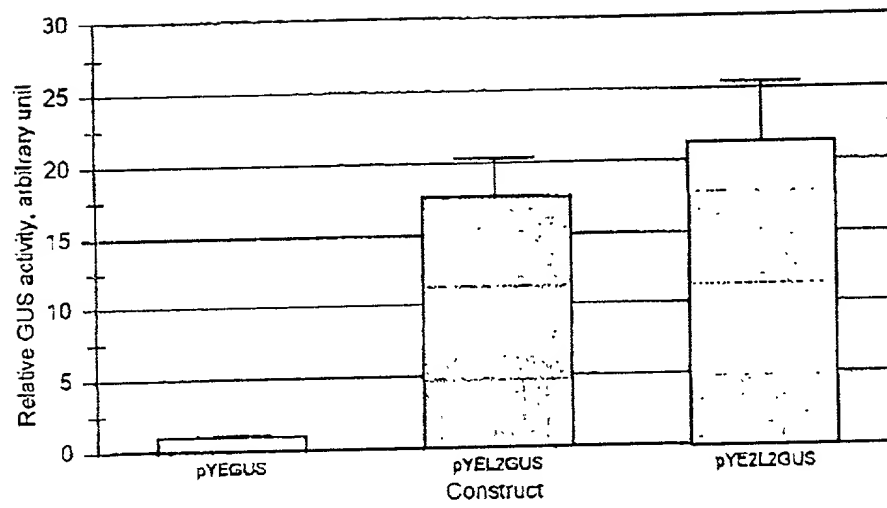
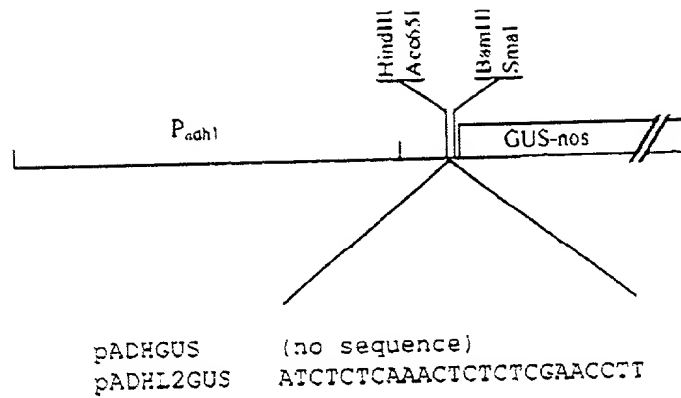


FIGURE 6L



# LEGEND:

- Vector sequence
- GUS reporter gene

FIGURE 6M







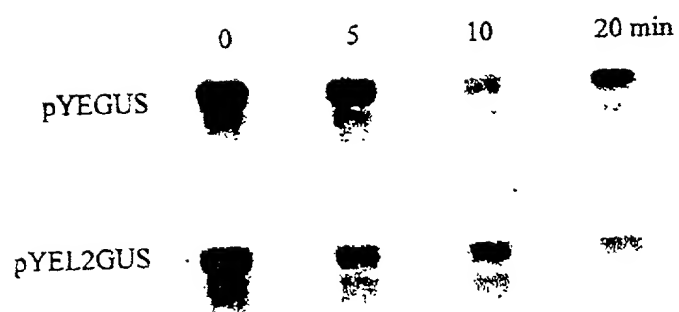


FIGURE 6P.1

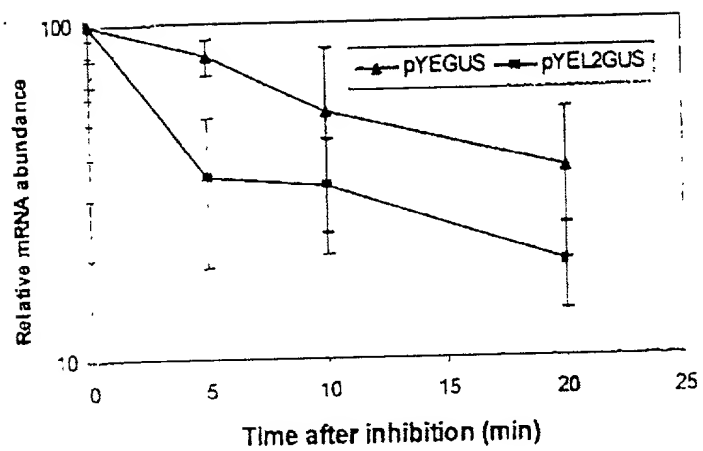


FIGURE 6P.2

## Enhanced tCUP Versions 1-3

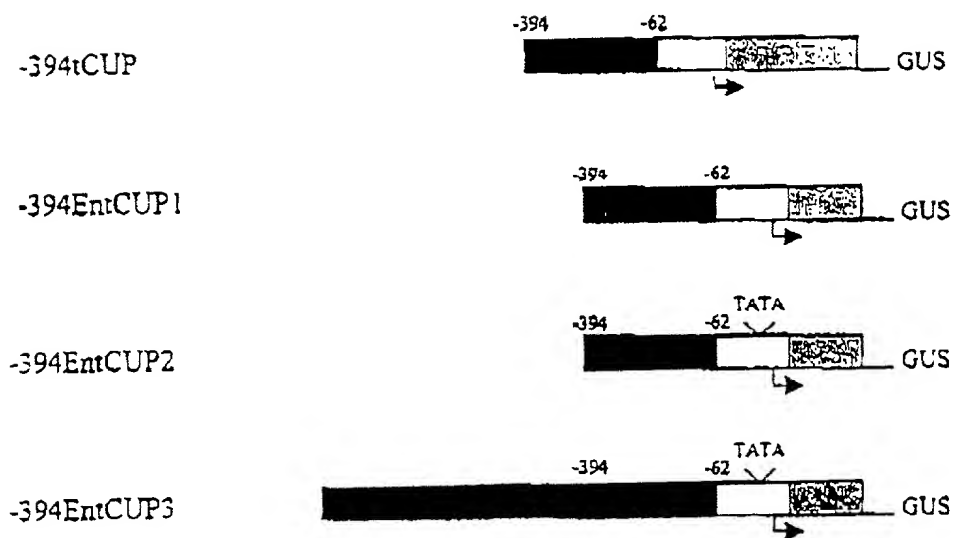
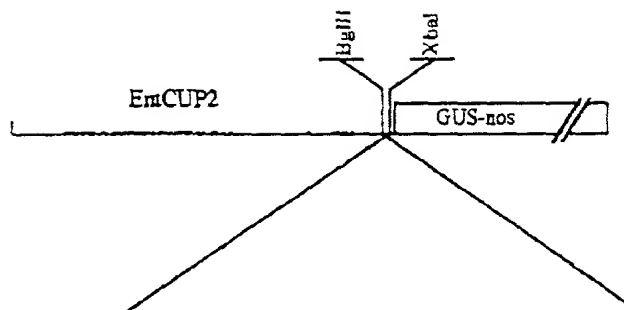


FIGURE 7A.1



pUCtCUP2(-N)GUS	(no sequence)
pUCtCUP2L1GUS	GGATCTATCCTCTTATCTCTCAA
pUCtCUP2L2GUS	ATCTCTCAAACTCTCTCGAACCTT
pUCtCUP2L3GUS	TTCCCTAAACCTAGCAG
pUCtCUP2L4GUS	ATCATCCTCACCTCAAAACCCACC
pUCtCUP2L5GUS	AGCCTCTCATCATCCTCACCTCAA
pUCtCUP2SCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pUCtCUP2SCAN7GUS	ATCTCTCAAACTCTCTCGAGAGTT
pUCtCUP2-2XL2GUS	ATCTCTCAAACTCTCTCGAACCTTTCTCTCAAACTCTCTCGAACCTT

FIGURE 7A.2

# Pooled Expression of GUS enhanced by L Series Fragments and enh-tCUP2 in Tobacco Transient Assay

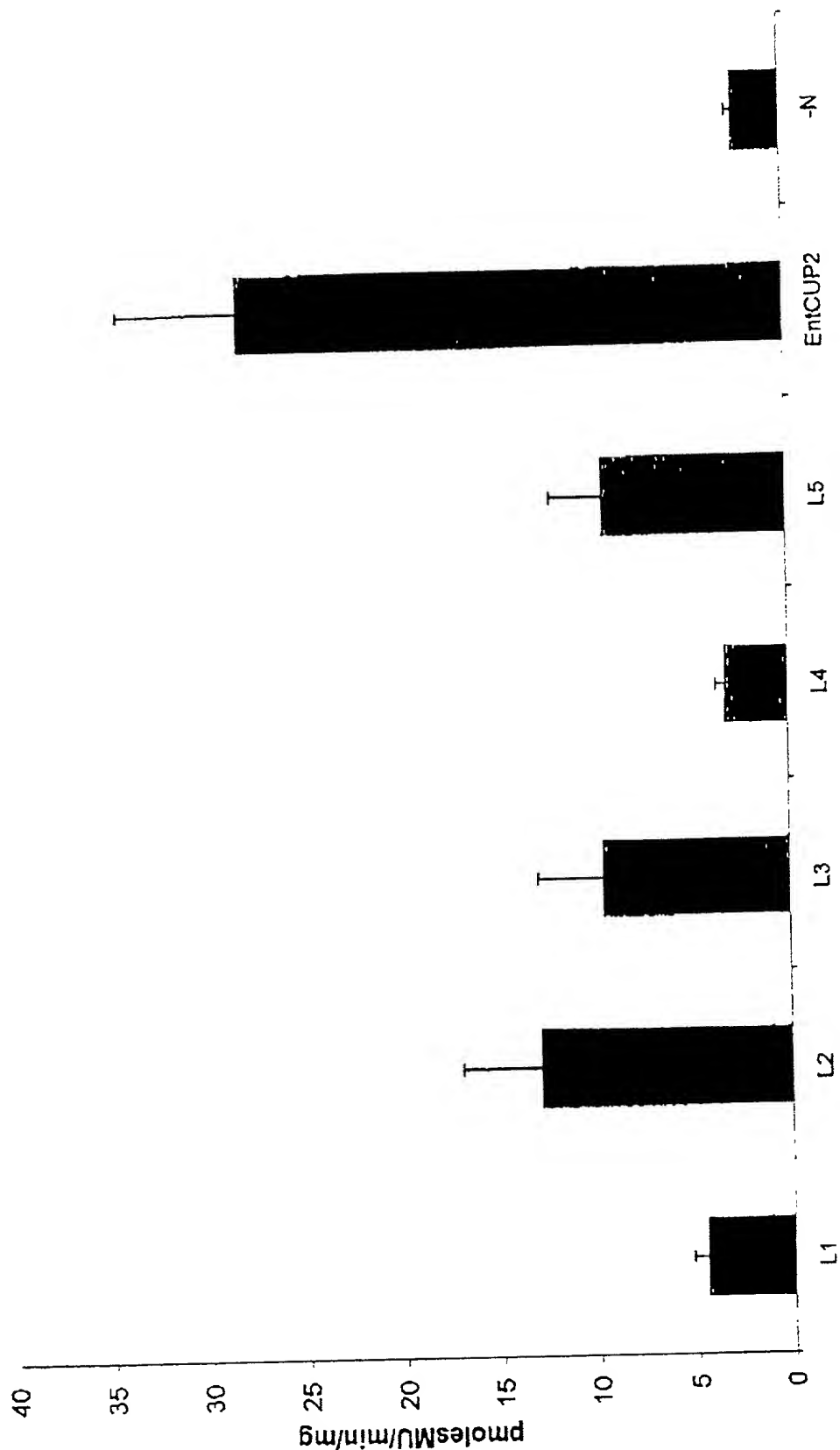


FIGURE 7B

Evaluation of tCUP leader element, L1, L2, L3, L4, and L5 on transient GUS gene expression in alfalfa suspension culture

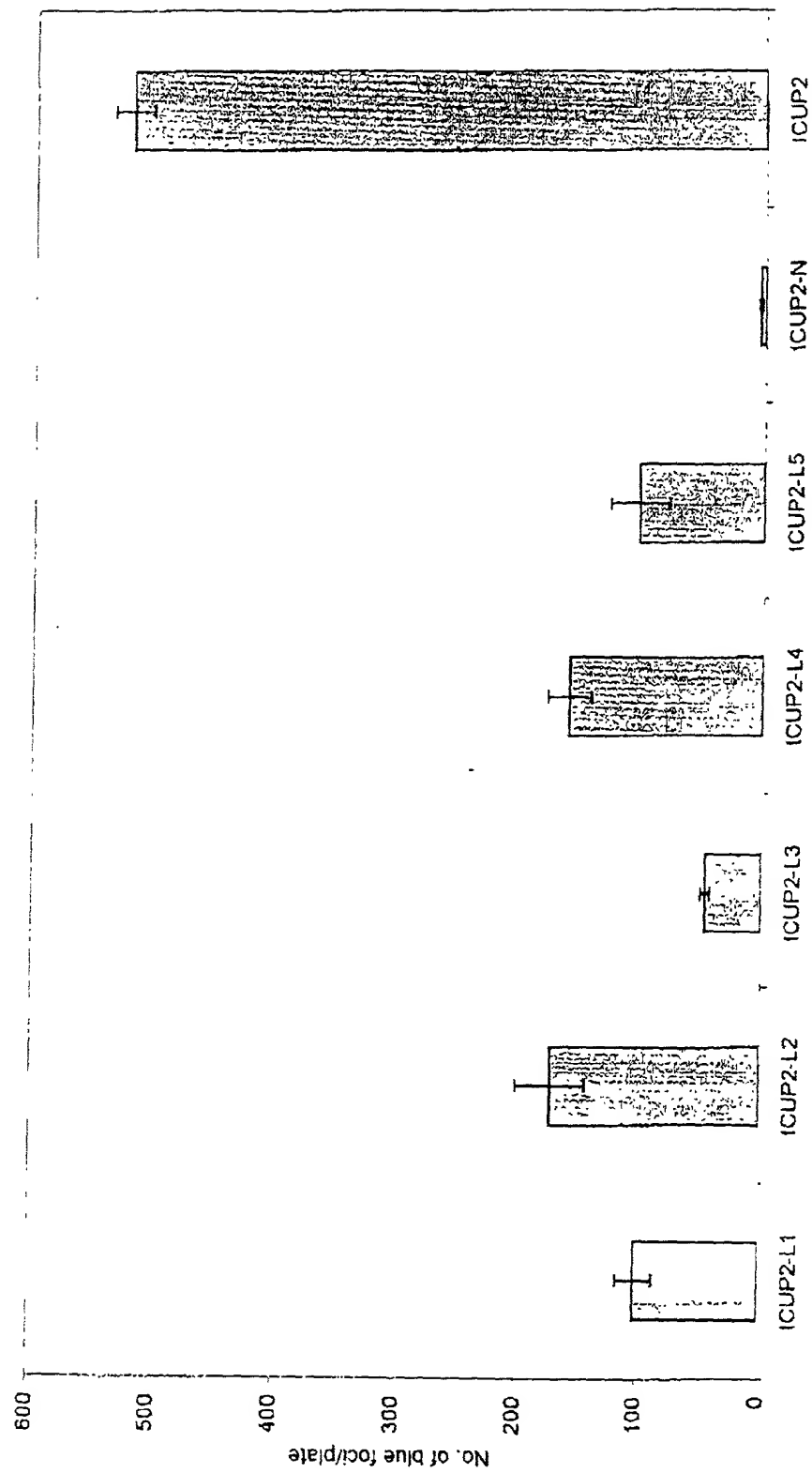


FIGURE 7C



# Evaluation of tCUP leader elements, L1, L2, L3, L4, and L5 on transient GUS gene expression in white spruce callus

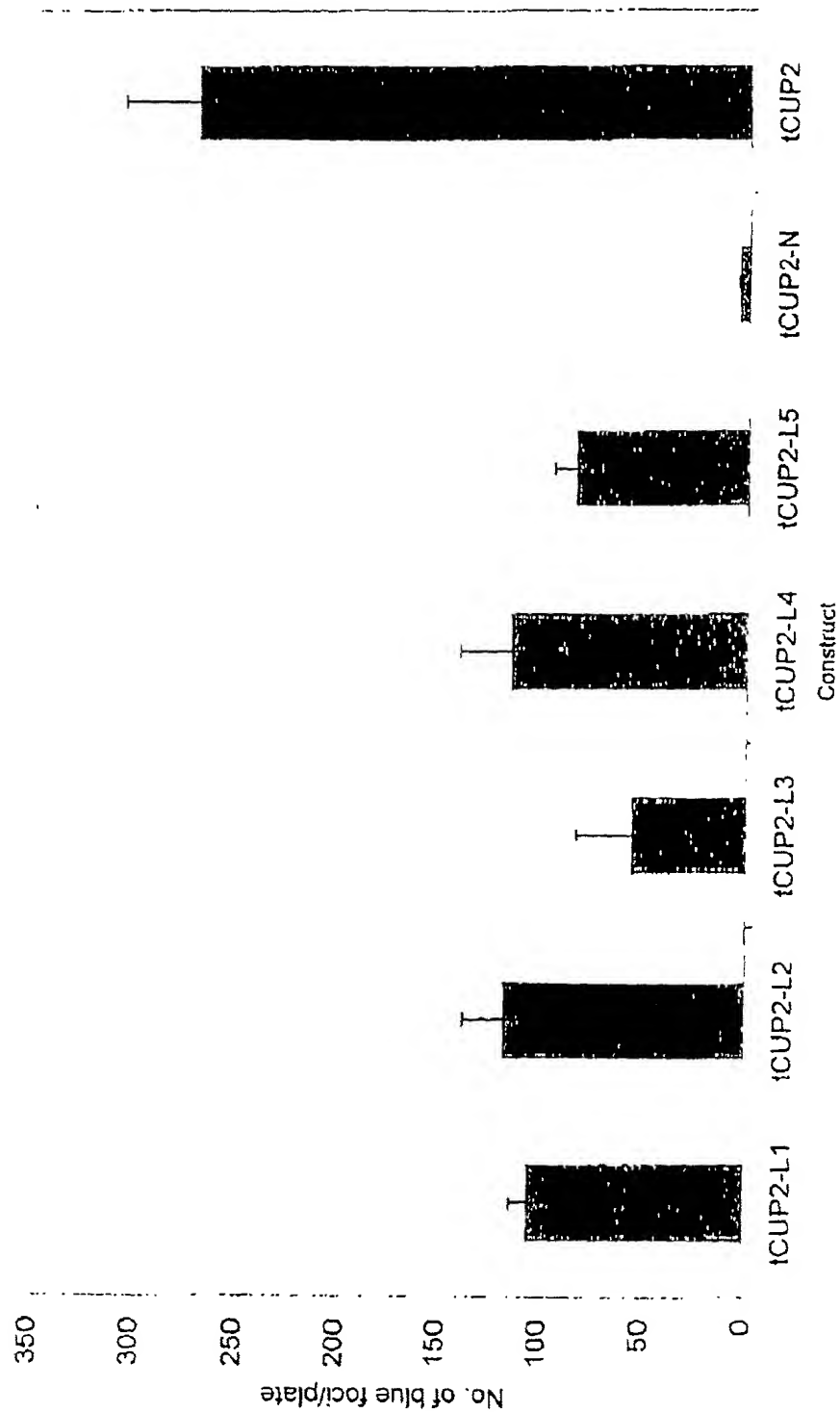
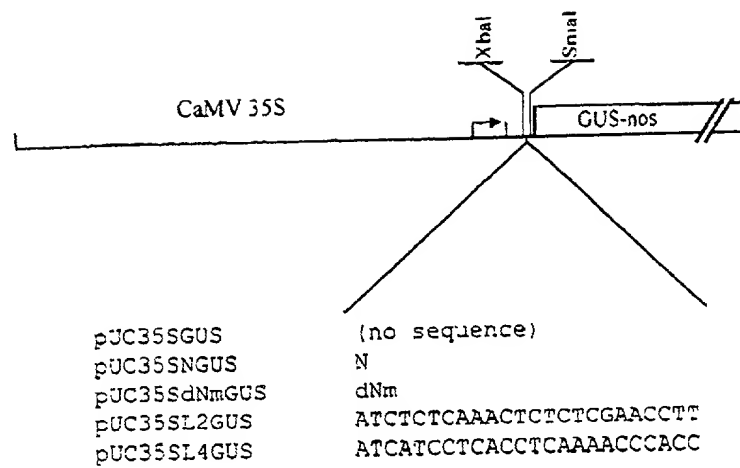


FIGURE 7D



# LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 8A

# **Stable Transformation of *Arabidopsis* with GUS enhanced by L-series constructs and the 35S promoter**

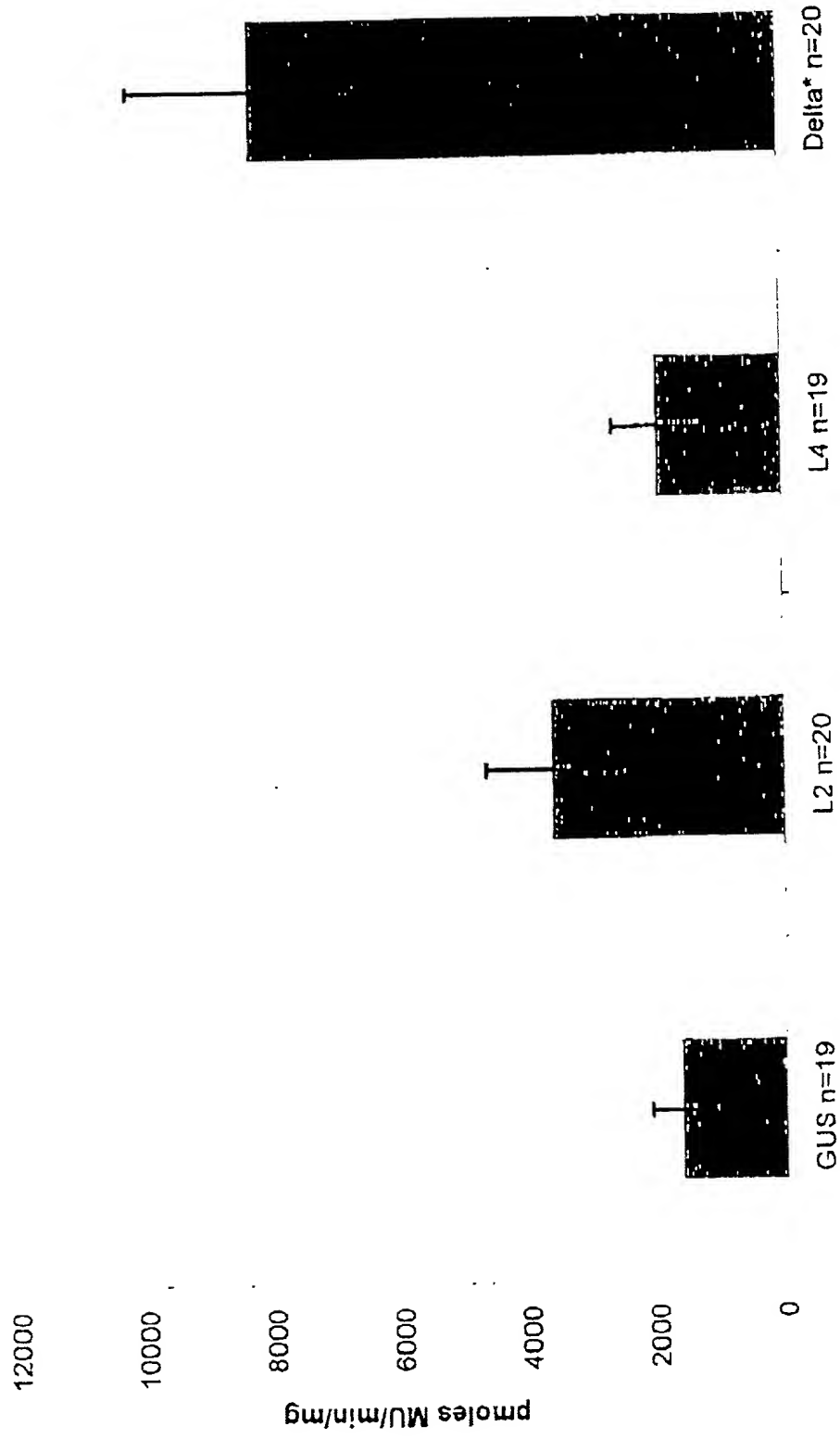


FIGURE 8B

Effect of L2 & L4 on 35S Pea Protoplast Expression

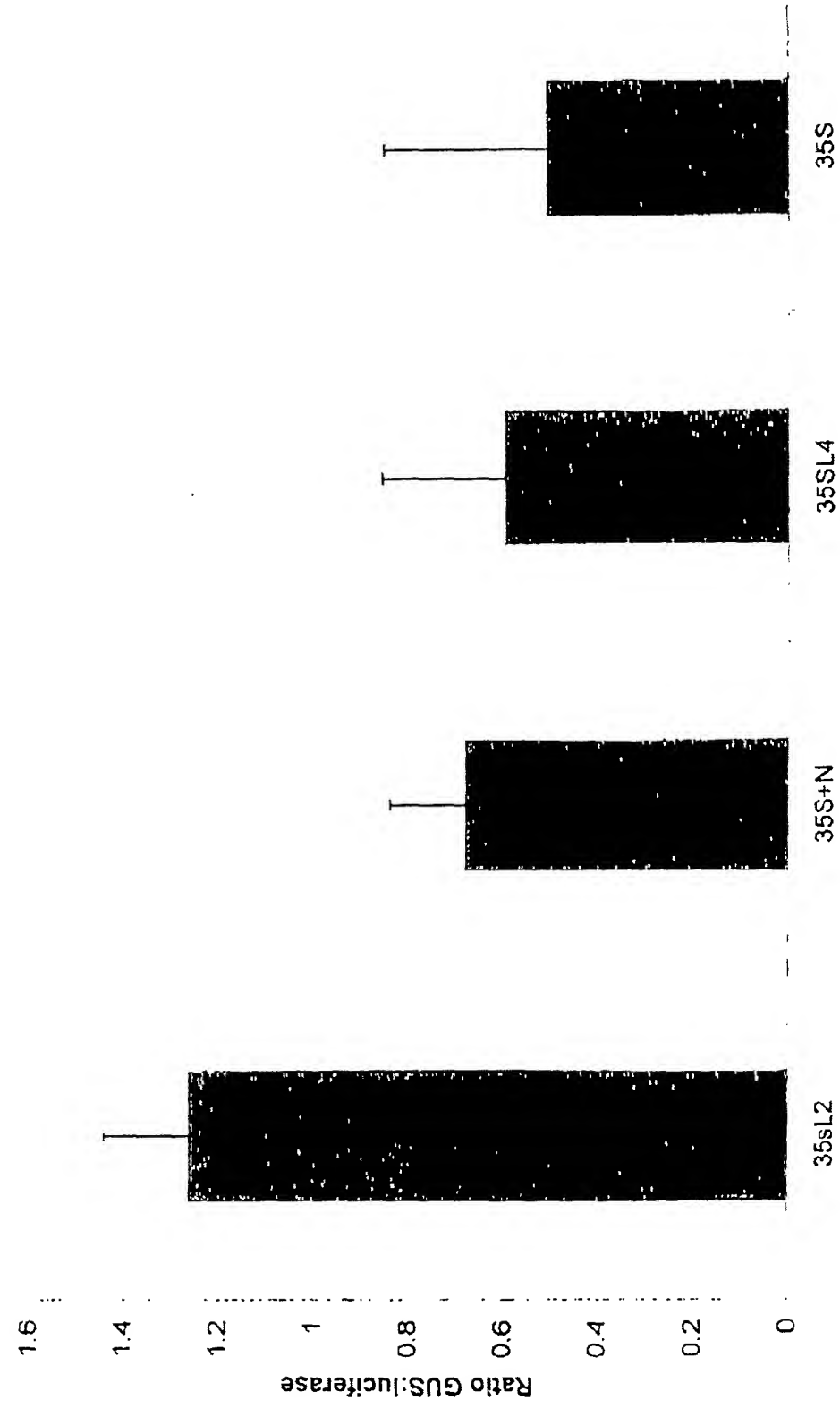


FIGURE 8C

### Effects of L2 and L4 on 35S Tobacco Transient Assay

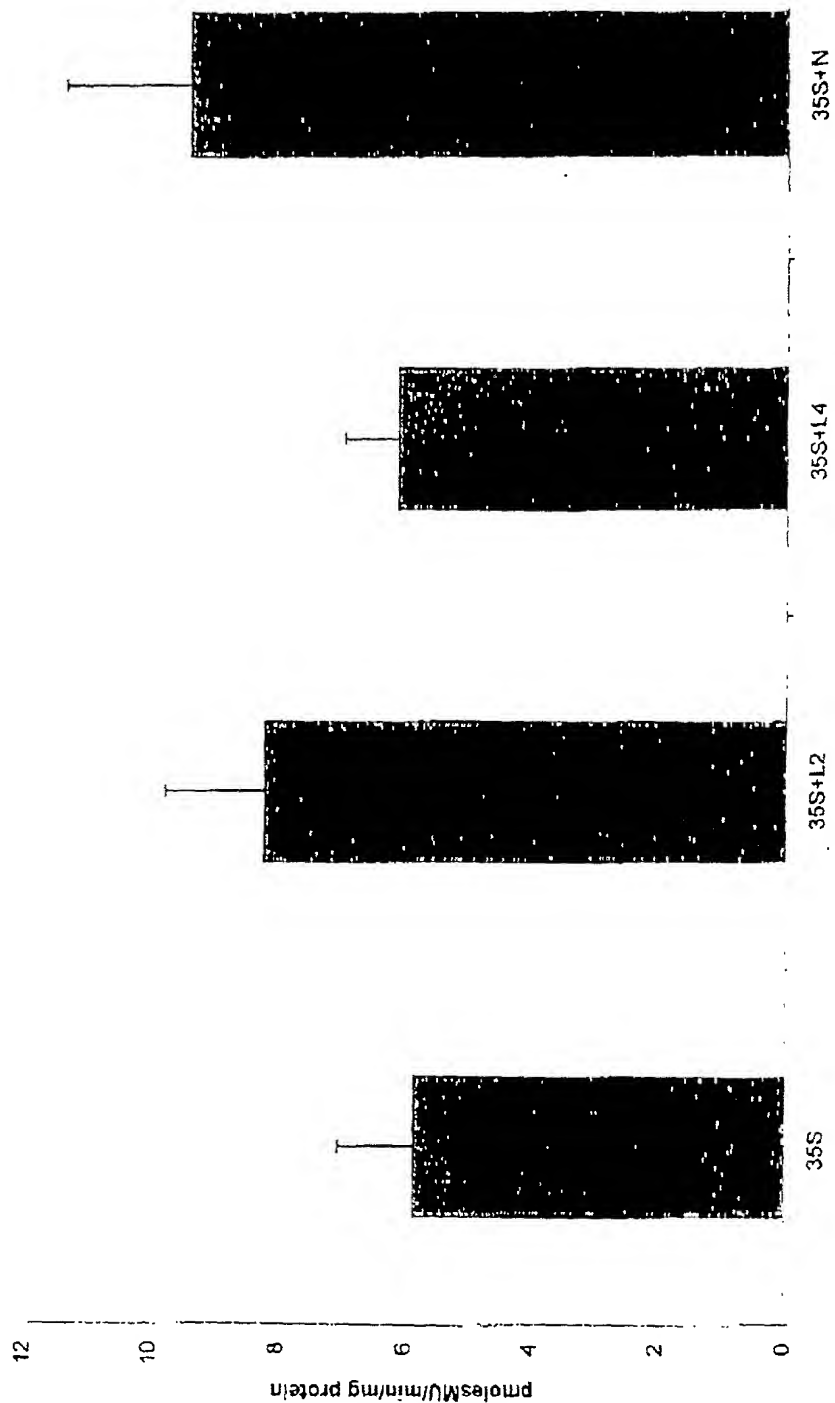


FIGURE 8D

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterologous promoter (35S) in a transient GUS gene expression in alfalfa suspension culture

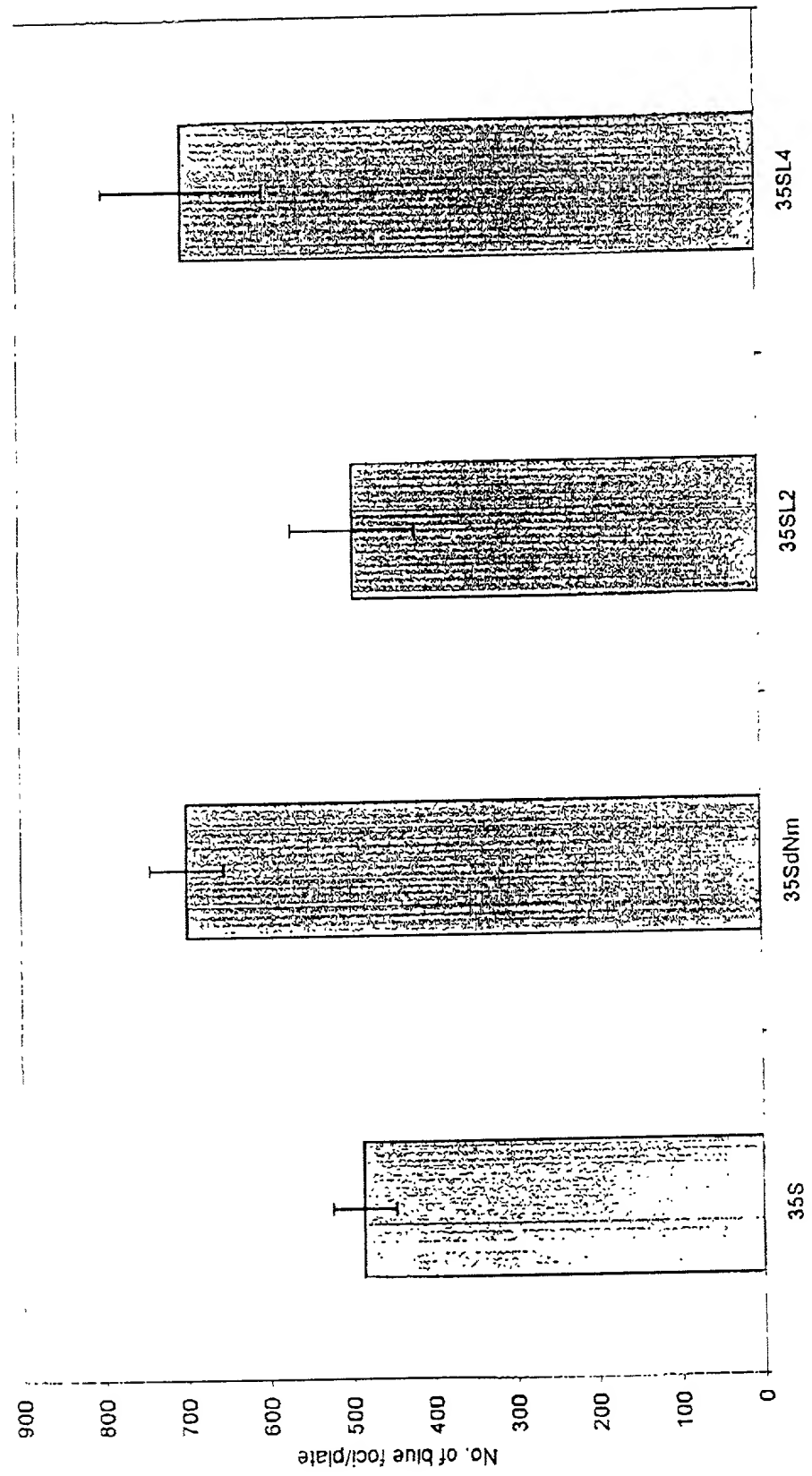


FIGURE 8E

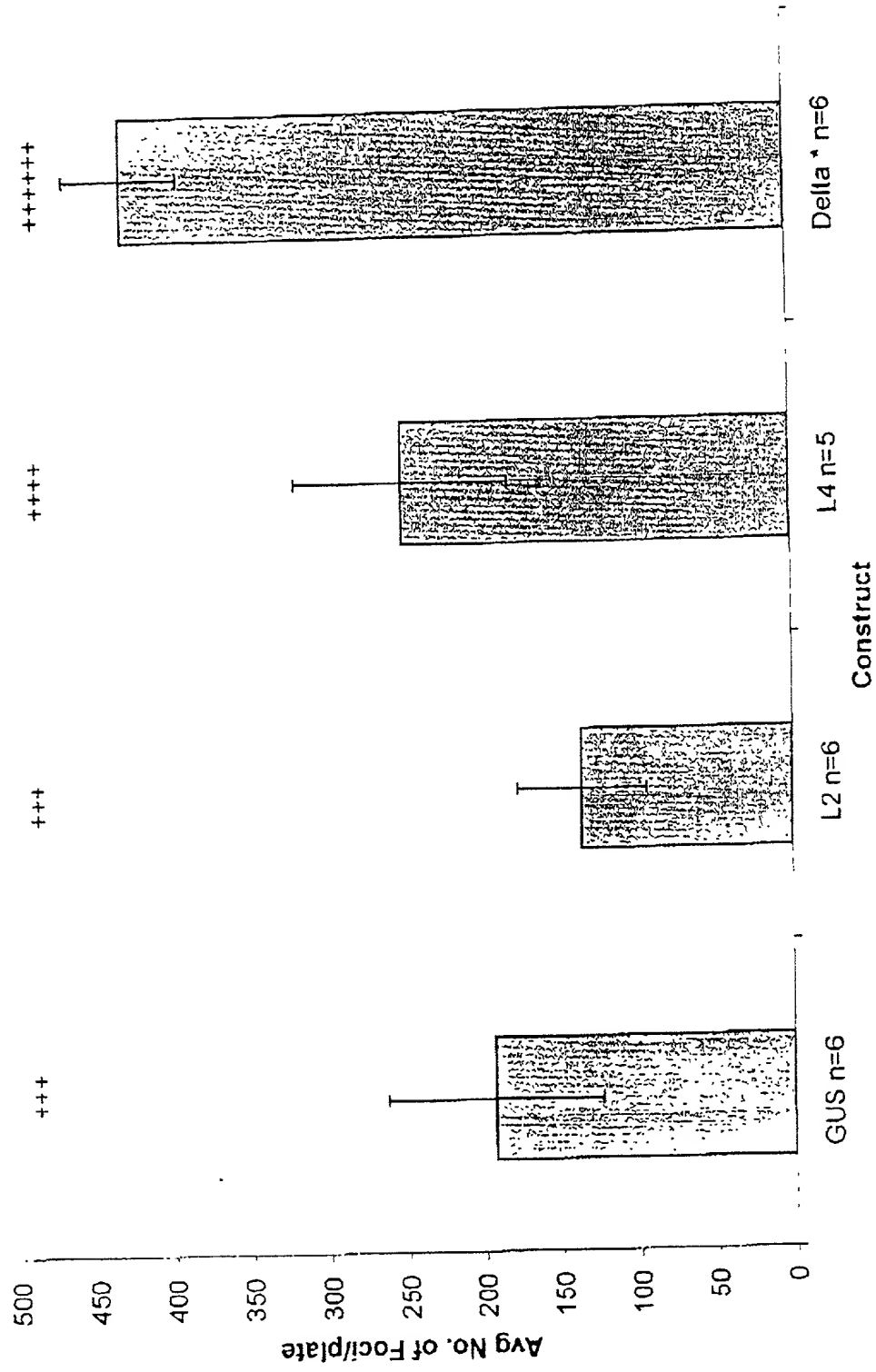


FIGURE 8F

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterologous promoter (35S) in a transient GUS gene expression in white spruce callus

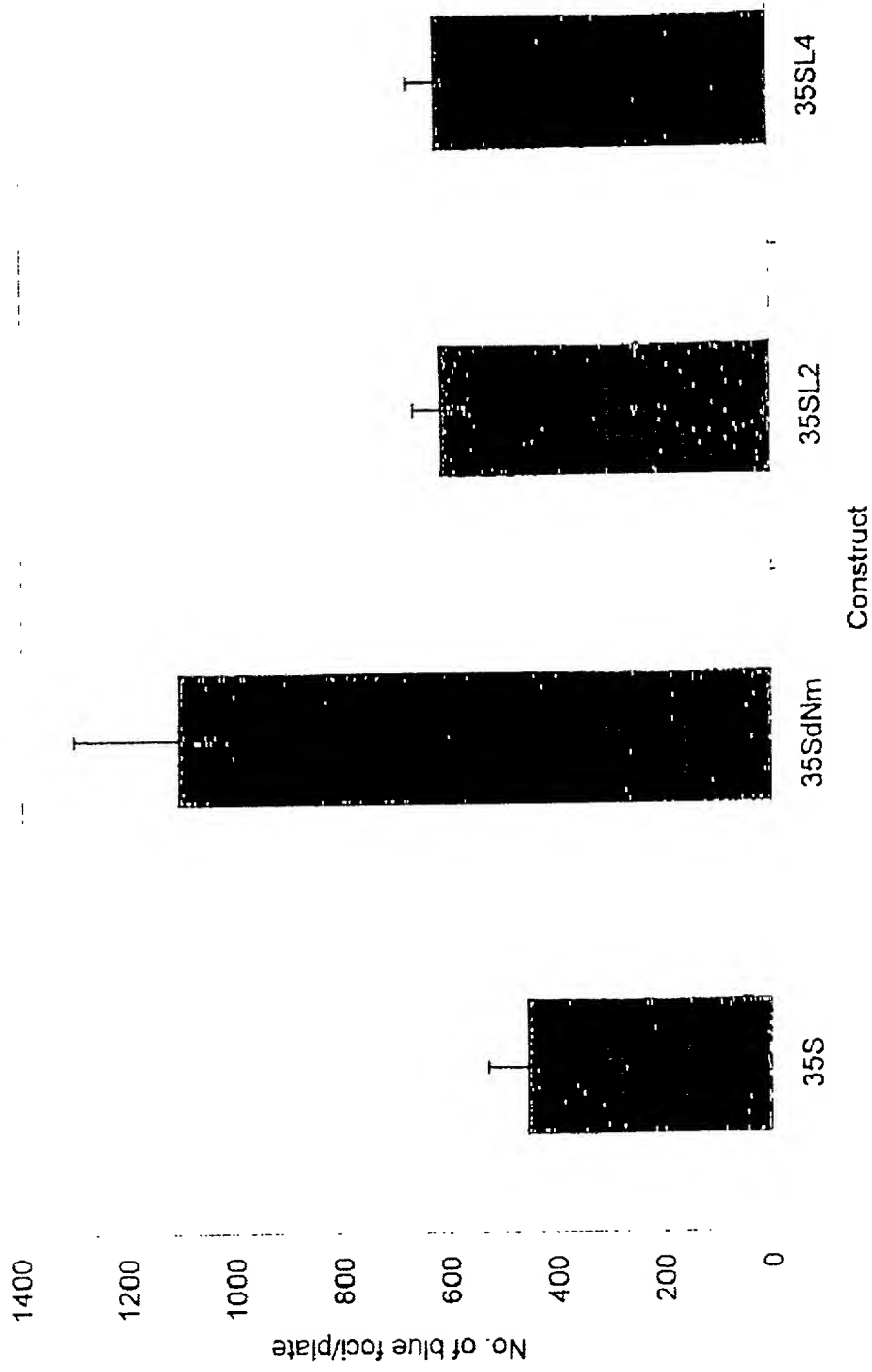
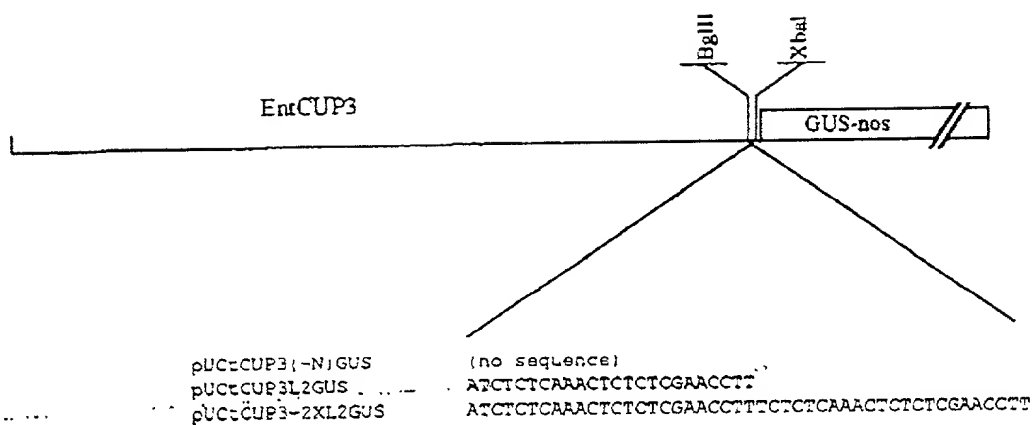


FIGURE 8G





# LEGEND:

- Vector sequence
- GUS reporter gene

FIGURE 9A

# GUS Expression of L2 Scan mutations and enh-tCUP2 in Tobacco Transient Assay

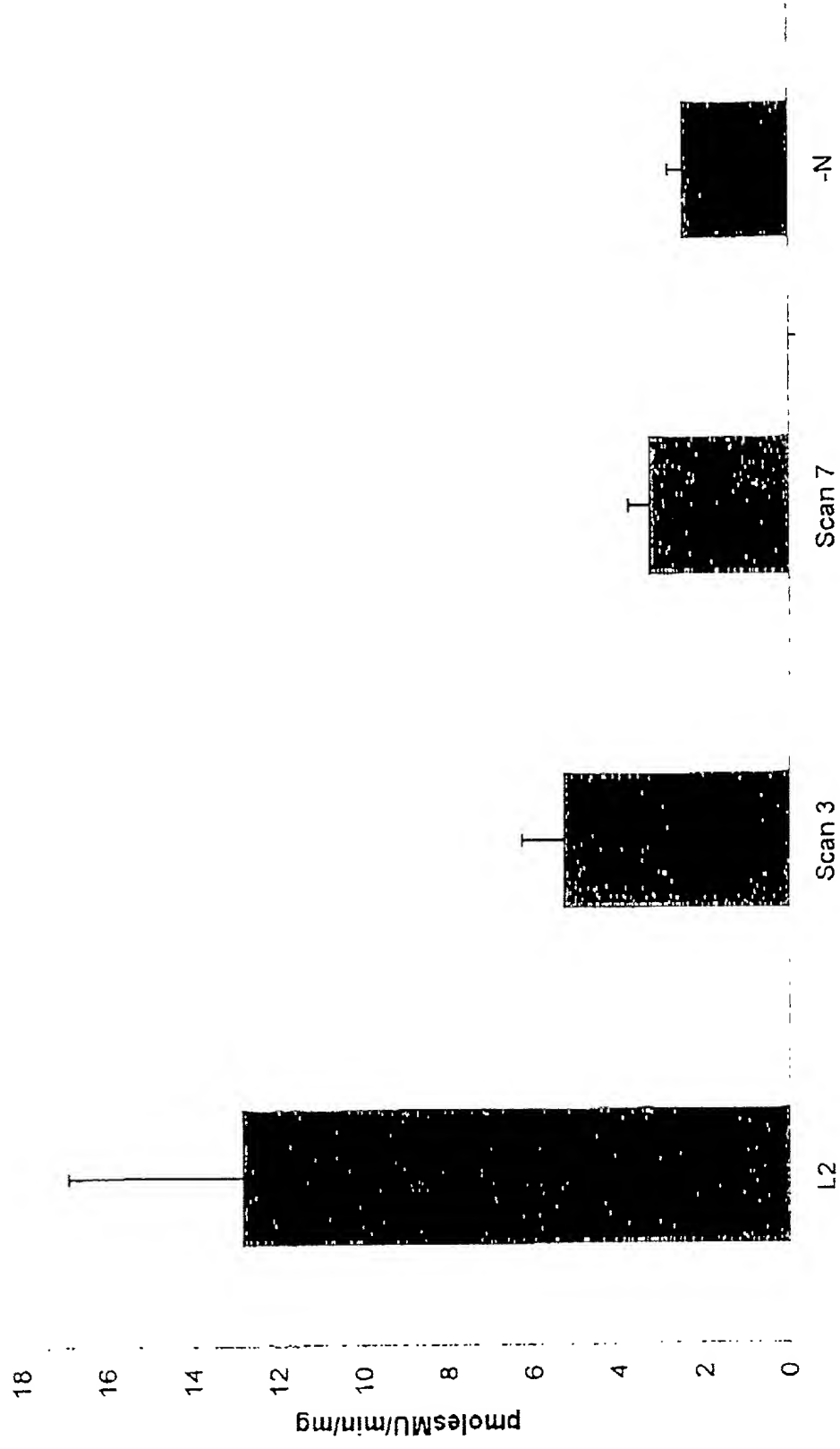


FIGURE 9B

# **Stable Transformation of *Arabidopsis* with GUS enhanced by L2 and 2XL2 constructs and the enh-tCUP2 and enh-tCUP3 promoter**

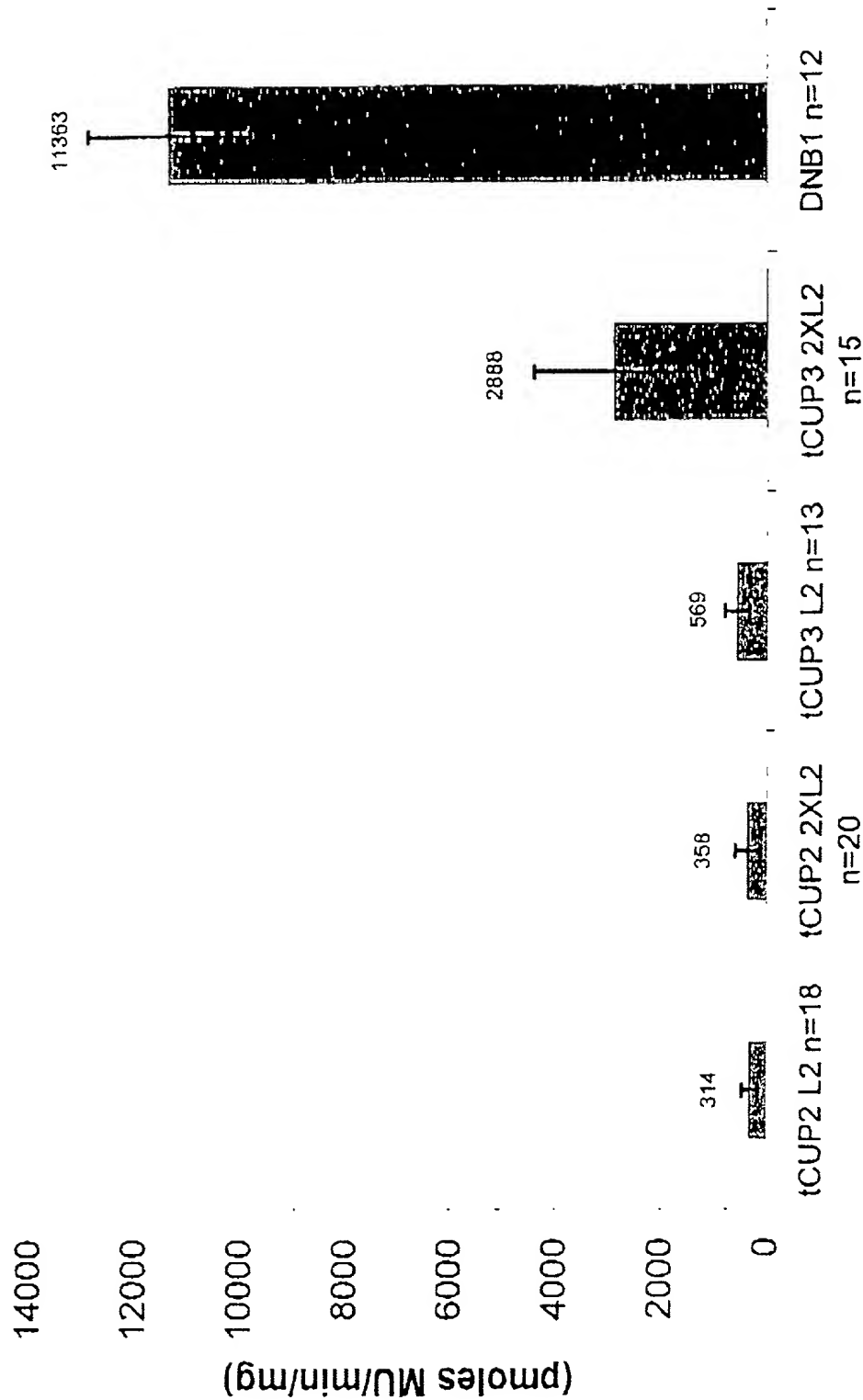


FIGURE 9C

# Tobacco leaf bombardment of Enhanced tCUP vectors with L2

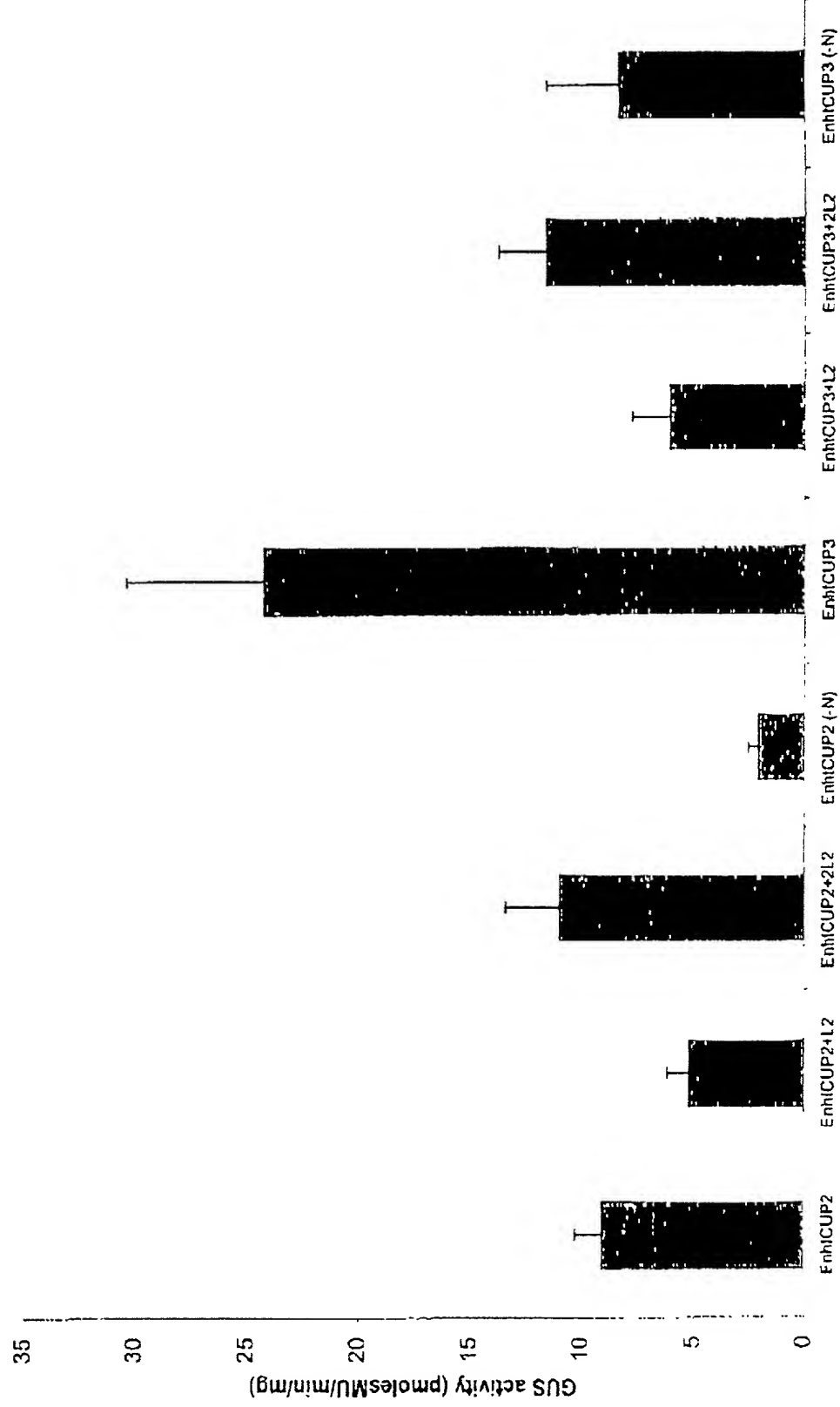


FIGURE 9D

Evaluation of ICUP leader element, L2, on transient GUS gene expression in alfalfa suspension culture

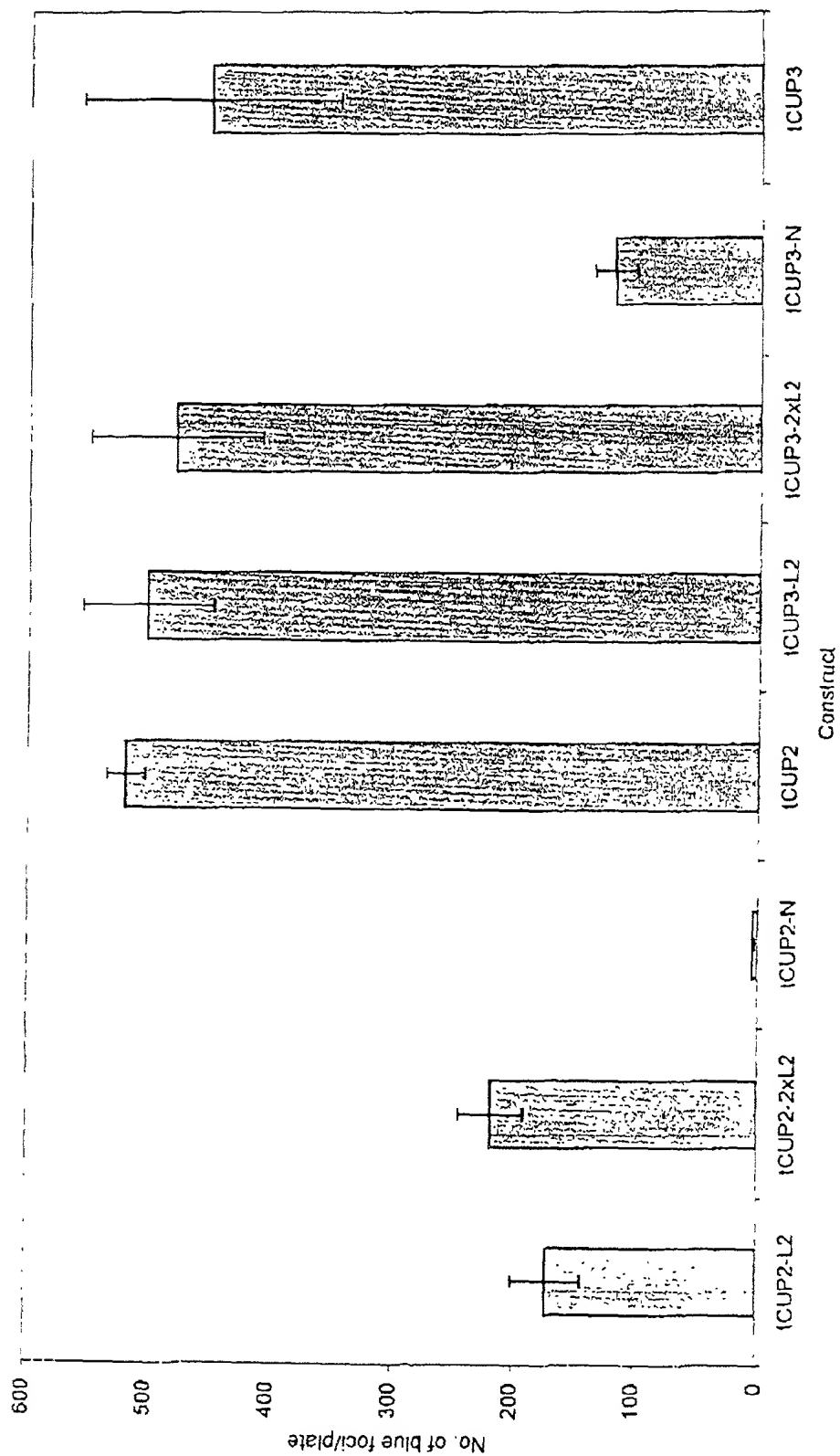
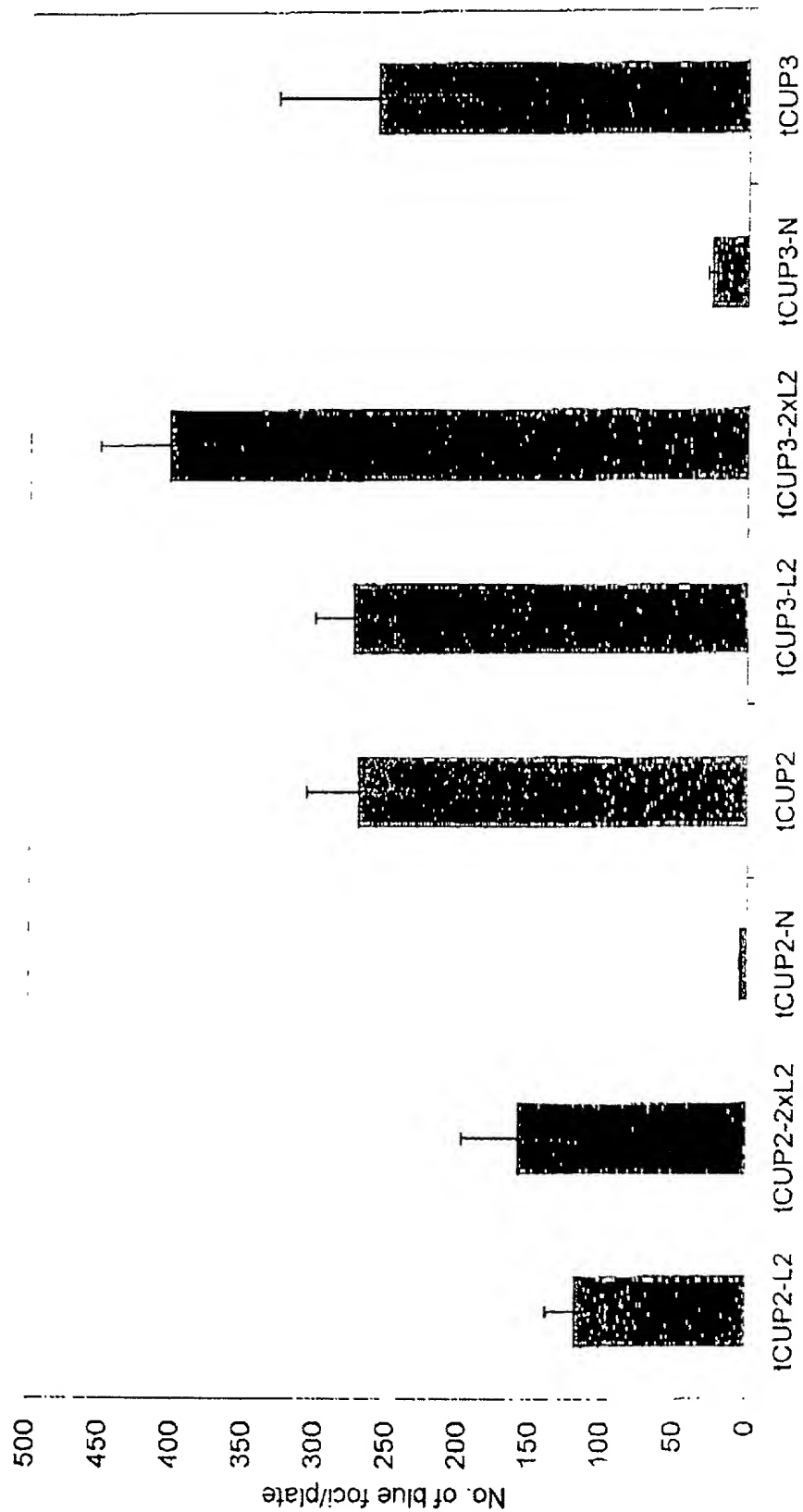


FIGURE 9E

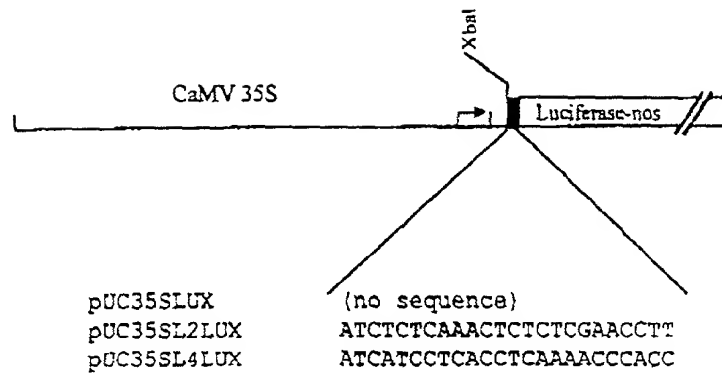
2005 120 2004 2004

Evaluation of tCUP leader element, L2, on transient GUS gene expression in  
white spruce callus



Construct

FIGURE 9F



LEGEND:

- Vector sequence
- ▭ Luciferase reporter gene
- █ L2 or L4
- ➞ Start of transcription

FIGURE 10A

Analysis of L2 and L4 in E. Coli using a luciferase reporter system

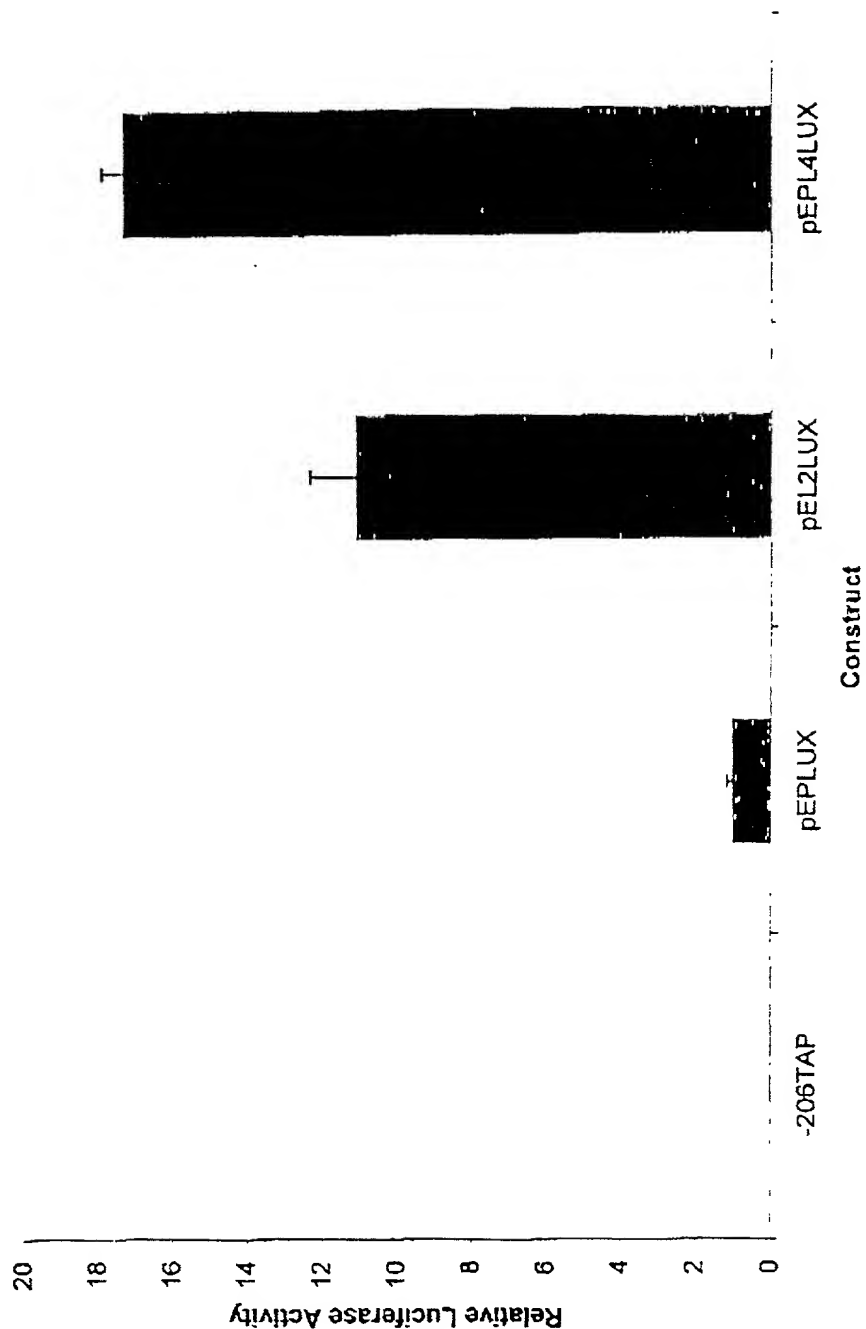


FIGURE 10B



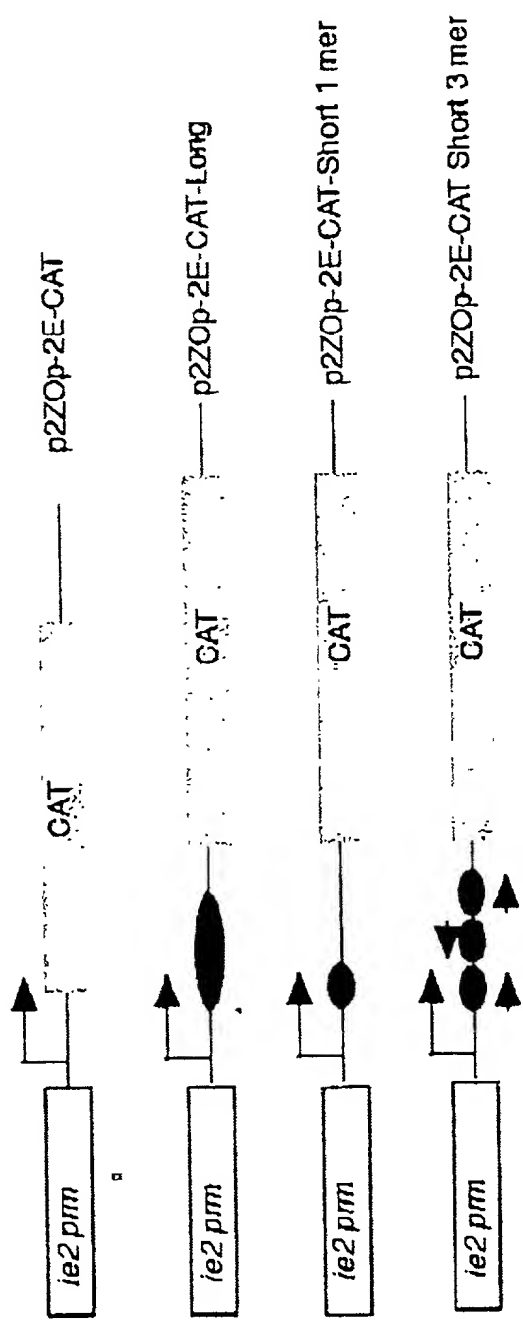


FIGURE 11A

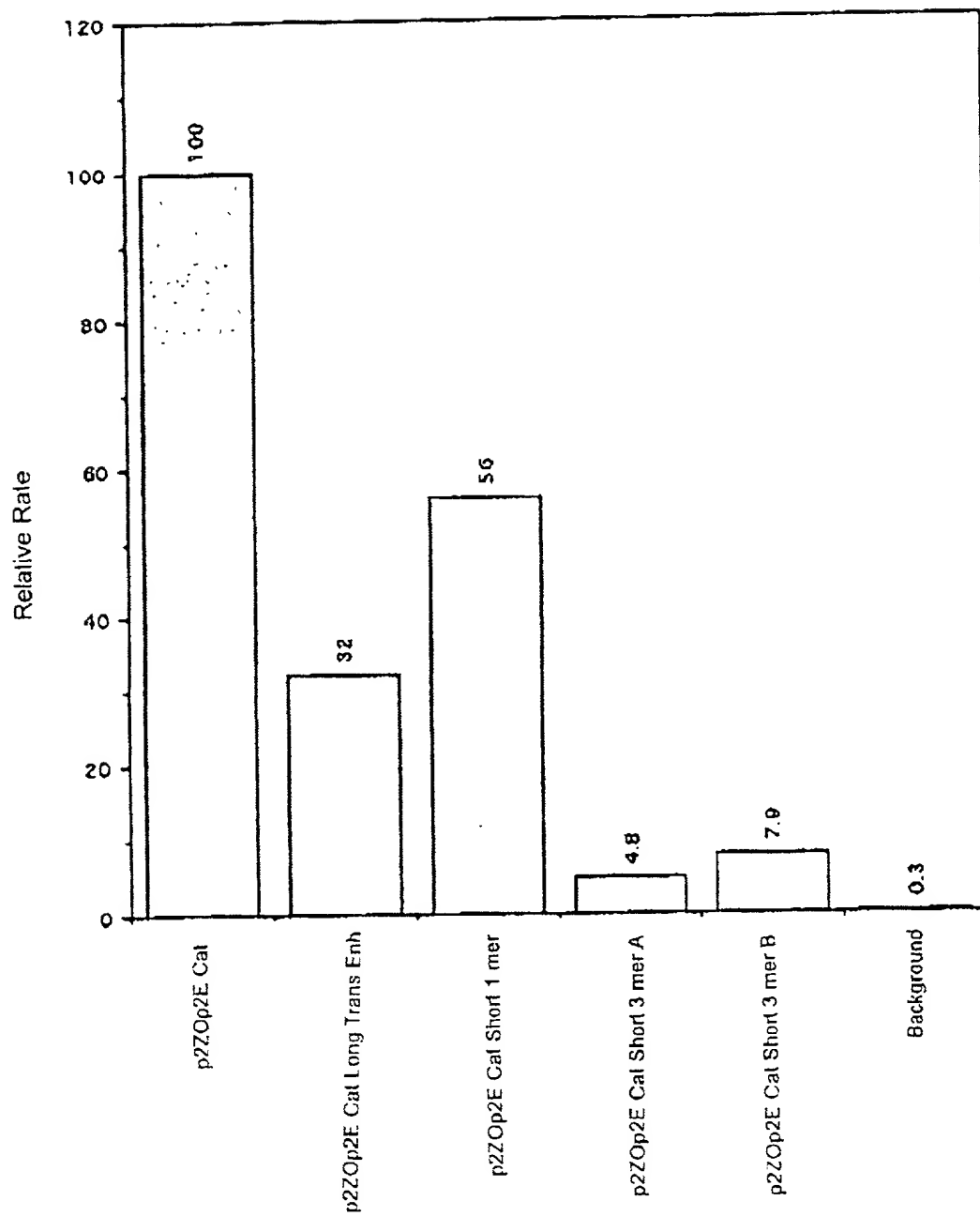


FIGURE 11B